Amendments

In the Specification:

Please amend the fourth full paragraph on page 7 as follows:

In another preferred embodiment, the amino acid linker is selected from the group consisting of (a) CGG; (b) N-terminal gamma 1-linker; (c) N-terminal gamma 3-linker; (d) Ig hinge regions; (e) N-terminal glycine linkers; (f) $(G)_kC\ (G)_n$ with n=0-12 and k=0-5; (g) N-terminal glycine-serine linkers; (h) $(G)_kC(G)_m(S)l(GGGGS)_n$ with n=0-3, k=0-5, m=0-10, l=0-2 (SEQ ID NO:424); (i) GGC; (k) GGC-NH2; (l) C-terminal gamma 1-linker; (m) C-terminal gamma 3-linker; (n) C-terminal glycine linkers; (o) $(G)_nC(G)_k$ with n=0-12 and k=0-5; (p) C-terminal glycine-serine linkers; (q) $(G)_m(S)_l(GGGGS)_n(G)_oC(G)_k$ with n=0-3, k=0-5, m=0-10, l=0-2, and o=0-8 (SEQ ID NO:425).

Please amend the first two descriptions of figures on page 11 as follows:

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A-1C Modular eukaryotic expression vectors for expression of antigens according to the invention (FIG. 1A:SEQ ID NO:426; FIG. 1B:SEQ ID NO:427; FIG. 1C:SEQ ID NO:428);

FIG. 2A-2C Cloning, expression and coupling of resistin to Qβ capsid protein (FIG. 2A, SEQ ID NO:429; FIG 2B, SEQ ID NO:430);

Please amend the third and fourth paragraphs on page 22 as follows:

Self antigen : As used herein, the tem "self antigen" refers to proteins encoded by the host's DNA and products generated by proteins or RNA encoded by the host's DNA are defined as self. In addition, proteins that result from a combination of two or several self-

molecules or that represent a fraction of a self-molecule and proteins that have a high homology [[two]]to self-molecules as defined above (>95%)may also be considered self.

Temperature-sensitive: As used herein, the phrase "temperature-sensitive" refers to an enzyme which readily catalyzes a reaction at one temperature but catalyzes the same reaction slowly or not at all at another temperature. An example of a temperature-sensitive enzyme is the replicase protein encoded by the pCYTts vector, which has readily detectable replicase activity at temperatures below 34°C and has low or undetectable activity at 37[[']] °C.

Please amend the specification from the second full paragraph on page 49 to the first paragraph on page 50 as follows:

In some embodiments, engineering of a second attachment site onto the antigen requires the fusion of an amino acid linker containing an amino acid suitable as second attachment site according to the disclosures of this invention. In a preferred embodiment, the amino acid is cysteine. The selection of the amino acid linker will be dependent on the nature of the self-antigen, on its biochemical properties, such as pI, charge distribution, glycosylation. In general, flexible amino acid linkers are favored. Examples of amino acid linkers are the hinge region of Immunoglobulins, glycine serine linkers $(GGGGS)_n$ (SEQ ID NO:407), and glycine linkers $(G)_n$ all further containing a cysteine residue as second attachment site and optionally further glycine residues. (In the following are examples of said amino acid linkers :

N-terminal gamma1: CGDKTHTSPP (SEQ ID NO:408)

C-terminal gamma 1: DKTHTSPPCG (SEQ ID NO:409)

N-terminal gamma 3: CGGPKPSTPPGSSGGAP (SEQ ID NO:410)

C-terminal gamma 3: PKPSTPPGSSGGAPGGCG (SEQ ID NO:411)

N-terminal glycine linker: GCGGGG (SEQ ID NO:412)

C-terminal glycine linker: GGGGCG (SEQ ID NO:413))

For peptides, GGCG (SEQ ID NO:414) linkers at the C-terminus of the peptide, or CGG at its N-terminus have shown to be useful. In general, glycine residues will be inserted between bulky amino acids and the cysteine to be used as second attachment site.

Please amend the first full paragraph on page 56 as follows:

Thus, in another preferred embodiment of the invention, the antigen or antigenic determinant is a peptide derived from the VEGFR-II contact site. This provides a composition and a vaccine composition in accordance with the invention, which may have antiangiogenic properties useful for the treatment of cancer. Inhibition of tumor growth in mice using sera specific for VEGFR-2 has been demonstrated (Wei, YQ., Wang, QR., Zhao, X., Yang, L., Tian, L., Lu, Y., Kang, B., Lu, CJ., Huang, MJ., Lou, YY., Xiao, F., He, QM., Shu, JM., Xie, XJ., Mao, YQ., Lei, S., Luo, F., Zhou, LQ., Liu, CE., Zhou, H., Jiang, Y., Peng, F., Yuan, LP., Li, Q., Wu, Y., Liu, JY. (2000) Nature Medicine 6, 1160-1165). Therefore, further preferred antigenic determinants suitable for inventive compositions and antiangiogenic vaccine compositions in accordance with the invention comprise either the human VEGFR-II derived peptide with the sequence CTARTELNVGIDFNWEYPSSKHQHKK (SEQ ID NO:351), and/or the murine VEGFR-II derived peptide having the sequence CTARTELNVGLDFTWHSPPSKSHHKK (SEQ ID NO:352), and/or the relevant extracellular globular domains 1-3 of the VEGFR-II.

Please amend the first and second full paragraphs on page 57 as follows:

In another preferred embodiment of the invention, the antigen or antigenic determinant is an angiotensin peptide or a fragment thereof. The term "angiotensin peptide" as used herein, shall encompass any peptide comprising the sequence, or fragments thereof,

of angiotensinogen, angiotensin I or angiotensin II. The sequences are as follows: Angiotensinogen: DRVYIHPFHLVIHN (SEQ ID NO:353); Angiotensin I: DRVYIHPFHL (SEQ ID NO:354); Angiotensin II: DRVYIHPF (SEQ ID NO:355). Typically, one or more additional amino acids are added either at the C- or at the N-terminus of the angiotensin peptide sequences. The sequence of the angiotensin peptides corresponds to the human sequence, which is identical to the murine sequence. Therefore, immunization of a human or a mouse with vaccines or compositions, respectively, comprising such angiotensin peptides as antigenic determinant in accordance with the invention, is a vaccination against a self-antigen. Those additional amino acids are, in particular, valuable for an oriented and ordered association to the core particle.

Preferably, the angiotensin peptide has an amino acid sequence selected from the group consisting of a) the amino acid sequence of CGGDRVYIHPF (SEQ ID NO:380); b) the amino acid sequence of CGGDRVYIHPFHL (SEQ ID NO:381); c) the amino acid sequence of DRVYIHPFHLGGC (SEQ ID NO:382); and d) the amino acid sequence of CDRVYIHPFH (SEQ ID NO:383).

Please amend the specification from the beginning of page 80 to the end of the first paragraph on page 81 as follows:

In a further preferred embodiment, the inventive composition comprises an amino acid linker containing a free cysteine and being added to the N-terminus of the sequence corresponding to the processed form of lymphotoxin-ß, or inserted between the N-terminus of the sequence corresponding to the mature form of the protein, and the signal peptide, C-terminally to the signal peptide. In further preferred embodiments of the invention, the extracellular part of lymphotoxin-ß is expressed as a fusion protein either with Glutathione-S-transferase, fused N-terminally to lymphotoxin-ß, or with a 6 histidine-tag followed by a

myc-tag, fused again N-terminally to the extracellular part of lymphotoxin-ß. An amino acid spacer containing a protease cleavage site as well as a linker sequence containing a free cysteine as attachment site, C-terminally to the protease cleavage site, are fused to the N-terminus of the sequence of the extracellular part of lymphotoxin-ß. Preferably, the extracellular part of lymphotoxin-ß consists of fragments corresponding to amino acids 49-306 or 126-306 of lymphotoxin-ß. These specific compositions of the invention may be cloned and expressed in the pCEP-Pu eukaryotic vector. In further preferred embodiments, the inventive compositions comprise an amino acid linker containing a free cysteine residue suitable as second attachment site, and being fused to the C-terminus of lymphotoxin-ß or lymphotoxin-ß fragments. In a particularly favored embodiment, the amino acid sequence LACGG (SEQ ID NO:415), comprising the amino acid linker ACGG (SEQ ID NO:416) which itself contains a cysteine residue for coupling to VLPS and Pili is fused to the N-terminus of the extracellular part of lymphotoxin-ß: or of a fragment of the extracellular part of lymphotoxin-β, yielding the proteins human C-LT[[•]]$\beta_{49\text{-}306}$ (SEQ ID NO:346) and human C-LT[[•]]$\beta_{126\text{-}306}$ (SEQ ID NO:347) after cleavage with enterokinase of the corresponding fusion proteins expressed either in vector pCEP-SP-GST-EK or vector pCP-SP-his-myc-EK as described in EXAMPLE 3.

In a preferred embodiment, the antigen or antigenic determinant is the prion protein, fragments thereof and in particular peptides of the prion protein. In one embodiment the prion protein is the human prion protein. Guidance on how to modify human prion protein for association with the ~~epre~~ core particle is given throughout the application and in particular in EXAMPLE 7. Mouse prion protein constructs are disclosed, and human prion protein constructs can also be generated and have, for example, the sequence of SEQ ID NO:348. Further constructs comprise the whole human prion protein sequence, and other fragments of the human prion protein, which are further composition of the invention. Immunization

against prion protein may provide a way of treatment or prevention of ~~Creutzfeld-Jakob~~ Creutzfeldt-Jakob (variant form) or other prion-mediated diseases. Immunization using the compositions of the invention comprising the prion protein may provide a way of treatment against prion mediated diseases in other animals, and the corresponding sequences of bovine and sheep prion protein constructs are given in SEQ ID NO:349 and SEQ ID NO:350, respectively. The peptides of the human prion protein corresponding to the murine peptides described in EXAMPLE 8, and of amino acid sequence CSAMSRPIIHFGSDYEDRYYRENMHR ("human cprplong"; SEQ ID NO:356) and CGSDYEDRYYRENMHR ("human cprpshort"; SEQ ID NO:357) lead to preferred embodiments of the invention. These peptides comprise an N-terminal cysteine residue added for coupling to VLPs and Pili. Corresponding bovine and sheep peptides are CSAMSRPLIHFGNDYEDRYYRENMHR ("bovine cprplong"; SEQ ID NO:401) and CGNDYEDRYYRENMHR ("bovine cprpshort"; SEQ ID NO:402) CSAMSRPLIHFGNDYEDRYYRENMYR ("sheep cprplong"; SEQ ID NO:403) and CGNDYEDRYYRENMYR ("sheep cprpshort"; SEQ ID NO:404), all leading to embodiments of the invention.

Please amend the specification from the third full paragraph on page 81 to the second full paragraph on page 82 as follows:

MuTNFa peptide: the sequence CGG was added at the N-terminus of the epitope consisting of amino acid residues 22-32 of mature murine TNF-α: CGGVEEQLEWLSQR (SEQ ID NO:358).

3'TNF II peptide: the sequence GGC was fused at the C-terminus of the epitope consisting of amino acid residues 4-22 of mature murine TNF-α and glutamine 21 was

mutated to glycine. The sequence of the resulting peptide is: SSQNSSDKPVAHVVANHGVGGC (SEQ ID NO:359).

5'TNF II peptide: a cysteine residue was fused to the N-terminus of the epitope consisting of amino acid residues 4-22 of mature murine TNF-α and glutamine 21 was mutated to glycine. The sequence of the resulting peptide is: CSSQNSSDKPVAHVVANHGV (SEQ ID NO:360).

The corresponding human sequence of the 4-22 epitope is SSRTPSDKPVAHVVANPQAEGQ (SEQ ID NO:361). Like for the murine sequence a cysteine is, preferably, fused at the N-terminus of the epitope, or the sequence GGC is fused at the C-terminus of the epitope for covalent coupling to VLPs, bacteriophages or bacterial pili according to the invention. It is, however, within the scope of the present invention that other cysteine containing sequences are fused at the N- or C-termini of the epitopes. In general, one or two glycine residues are preferably inserted between the added cysteine residue and the sequence of the epitope. Other amino acids may, however, also be inserted instead of glycine residues, and these amino acid residues will preferably be small amino acids such as serine.

The human sequence corresponding to amino acid residues 22-32 is QLQWLNRRANA (SEQ ID NO:362). Preferably, the sequence CGG is fused at the N-terminus of the epitope for covalent coupling to VLPs or bacterial pili according to the invention. Other TNF-α epitopes suitable for using in the present invention have been described and are disclosed for example by Yone *et al.* (*J. Biol. Chem.*270: 19509-19515).

Please amend the third paragraph on page 87 as follows:

Assembly of the ordered and repetitive antigen array in the *JUN/FOS* embodiment is done in the presence of a redox shuffle. E2-*JUN* viral particles are combined with a 240 fold

molar excess of *FOS*-antigen or *FOS*-antigenic determinant for 10 hours at 4[[α]]±°C. Subsequently, the AlphaVaccine particle is concentrated and purified by chromatography (Example 16).

Please amend the specification beginning with the fourth full paragraph on page 95 and continuing onto page 96 as follows:

A modular system containing a free cysteine flanked by several glycines, a protease cleavage site and the constant region of the human IgG1 was generated as follows. pSec2/Hygro B (Invitrogen Cat. No. V910-20) was digested with Bsp120I and Hind III and ligated with the annealed oligonucleotides SU7 (SEQ ID NO:278) and SU8 (SEQ ID NO:279) leading to construct pSec-B-MCS. pSec-B-MCS was then digested with Nhe I and Hind III and ligated with the annealed oligonucleotides PH29 (SEQ ID NO:264) and PH30 (SEQ ID NO:265) leading to construct pSec 29/30. The construct pSec-FL-EK-Fc* was generated by a three fragment ligation of the following fragments; first pSec 29/30 digested with Eco RI and Hind III, the annealed oligonucleotides PH31 (SEQ ID NO:266) and PH32 (SEQ ID NO[[.]]±267) and the Bgl I/EcoRI fragment of a plasmid (pSP-Fc*-C1) containing a modified version of the human IgG1 constant region (for details of the hu IgG1 sequence see the sequence of the final construct pCep-Xa-Fc* see FIG. 1A-1C, SEQ ID NOS:426, 427 and 428, respectively). The complete sequence of pCep-Xa-Fc* is given in SEQ ID NO:283. The resulting construct was named pSec-FL-EK-Fc*. From this plasmid the linker region and the human IgG1 Fc part was excised by Nhe I, Pme I digestion and cloned into pCep-MCS digested with Nhe I and Pme I leading to construct pCep-FL-EK-Fc*. Thus a modular vector, was created where the linker sequence and the protease cleavage site, which are located between the Nhe I and Hind III sites, can easily be exchanged with annealed oligonucleotides. For the generation of cleavable fusion protein vectors pCep-FL-EK-Fc*

was digested with Nhe I and Hind III and the Factor Xa cleavage site N-terminally flanked with amino acids GGGGCG (SEQ ID NO:413) was introduced with the annealed ~~oligonuclotides~~ oligonucleotides PH35 (SEQ ID NO:268) and PH36 (SEQ ID NO:269) and the enterokinase site flanked n-terminally with GGGGCG (SEQ ID NO:413) was introduced with the annealed oligonucleotides PH39 (SEQ ID NO:272) and PH40 (SEQ ID NO:273) leading to the constructs pCep-Xa-Fc* (see FIG. 1A, SEQ ID NO:426) and pCep-EK-Fc* (see FIG. 1B, SEQ ID NO:427) respectively. The construct pCep-SP-EK-Fc* (see FIG. 1C, SEQ ID NO:428) which in addition contains a eukaryotic signal peptide was generated by a three fragment ligation of pCep-EK-Fc* digested Kpn I/ Bam HI, the annealed oligos PH41 (SEQ ID NO:274) and PH42 (SEQ ID NO:275) and the annealed oligos PH43 (SEQ ID NO:276) and PH44 (SEQ ID NO:277).

Please amend the third through sixth full paragraphs on page 97 as follows:

FIG. 1A-1C (SEQ ID NOS:426, 427 and 428, respectively) shows partial sequences of the different eukaryotic expression vectors used. Only the modified sequences are shown.

FIG 1A (SEQ ID NO:426): pCep-Xa-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence. The arrow indicates the cleavage site of the factor Xa protease.

FIG 1B (SEQ ID NO:427): pCep-EK-Fc*: the sequence is shown from the Bam HI site onwards and different features are shown above the translated sequence. The arrow indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG 1A.

FIG. 1C (SEQ ID NO:428): pCep-SP-EK-Fc*: the sequence is shown from the beginning of the signal peptide on and different features are shown above the translated sequence. The signal peptide sequence which is cleaved of by the signal peptidase is shown

in bold The arrow indicates the cleavage site of the enterokinase. The sequence downstream of the Hind III site is identical to the one shown in FIG 1A (SEQ ID NO:426).

Please amend the first and second full paragraphs on page 99 as follows:

pCep-mRes-Xa-Fc* and pCep-mRes-EK-Fc* constructs were then used to transfect 293-EBNA cells for the production of recombinant proteins as described in EXAMPLE 1, section B. The tissue culture supernatants were purified as described in EXAMPLE 1, section C. The purified proteins were then cleaved as described in EXAMPLE 1, section D. The resulting recombinant proteins were termed "resistin-C-Xa" or "Res-C-Xa" and "resistin-C-EK" or "Res-C-EK" according to the vector used (see FIG. 2A and FIG. 2B, SEQ ID NOS:429 and 430, respectively).

FIG. 2A and FIG. 2B (SEQ ID NOS:429 and 430, respectively) show sequence of recombinant mouse Resistin proteins used for expression and further coupling. Res-C-Xa (FIG. 2A, SEQ ID NO:429) and Res-C-EK (FIG. 2B, SEQ ID NO:430) are shown as a translated DNA sequences. The resistin signal sequence which is cleaved upon protein secretion by the signal peptidase is shown in italic. The amino acid sequences which result form signal peptidase and specific protease (factor Xa or enterokinase) cleavage are shown bold. The bold sequences correspond to the actual protein sequence which was used for coupling, i.e. SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282 corresponds to an alternative resistin protein construct, which can also be used for coupling to virus-like particles and pili in accordance with the invention.

Please amend the specification from the second full paragraph on page 101 to the second paragraph on page 107 as follows:

The extracellular part of mouse lymphotoxin-[[•]]β (LT-[[•]]β) was recombinantly expressed with a CGG amino acid linker at its N-terminus. The linker contained one cysteine for coupling to VLP. A long (aa 49-306) and a short version (aa 126-306) of the protein were fused at their N-terminus to either glutathione S-transferase (GST) or a ~~histidin-myc~~ histidine-myc tag for purification. An enterokinase (EK) cleavage-site was inserted for cleavage of the tag.

Construction of C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$.

Mouse LT[[•]]$\beta_{49\text{-}306}$ was amplified by PCR with oligos 5'LT[[•]]β and 3'LT[[•]]β from a mouse spleen cDNA library inserted into pFB-LIB. For the PCR reaction, 0.5 µg of each primer and 200 ng of the template DNA was used in the 50 [[•]]µl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM $MgSO_4$). The temperature cycles were as follows: 94°C for 2 minutes, followed by 25 cycles of 94°C (15 seconds), 68°C (30 seconds), 68°C (1 minute) and followed by 68°C for 10 minutes. The PCR product was phosphorylated with T4 Kinase and ligated into pEntry1A (Life technologies) which has been cut with *EcoR*V and has been dephosphorylated. The resulting plasmid was named pEntry1A-LT[[•]]$\beta_{49\text{-}306}$.

A second PCR reaction was performed with oligos 5'LT[[•]]βlong-*Nhe*I and 3'LT[[•]]βstop-*Not*I resp. 5'LT[[•]]βshort-*Nhe*I and 3'LT[[•]]βstop-*Not*I using pEntry1A-LT[[•]]$\beta_{49\text{-}306}$ as a template. Oligos 5'LT[[•]]βlong-*Nhe*I and 5'LT[[•]]βshort-*Nhe*I had an internal *Nhe*I site and contained codons for a Cys-Gly-Gly linker and 3'LT[[•]]βstop-*Not*I had an internal *Not*I site and contained a stop codon. For the second PCR reaction, 0.5 µg of each primer and 150 ng of the template DNA was used in the 50 [[•]]µl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM MgSO4). The temperature

cycles were as follows: 94°C for 2 minutes, followed by 5 cycles of 94°C (15 seconds), 50°C (30 seconds), 68°C (1 minute), followed by 20 cylces cycles of 94°C (15 seconds), 64°C (30 seconds), 68°C (1 minute) and followed by 68°C for 10 minutes.

The PCR products were digested with *Nhe*I and *Not*I and inserted into either pCEP-SP-GST-EK or pCEP-SP-his-myc-EK (Wuttke *et al. J. Biol. Chem. 276*: 36839-48 (2001)). Resulting plasmids were named pCEP-SP-GST-EK-C-LT[[•]]β$_{49-306}$, pCEP-SP-GST-EK-C-LT[[•]]β$_{126-306}$, pCEP-SP-his-myc-EK-C-LT[[•]]β$_{49-306}$, pCEP-SP-his-myc-EK-C-LT[[•]]β$_{126-306}$, respectively. GST stands for glutathione-S-transferase, EK for enterokinase, his for a hexahistidine tag and myc for anti c-myc epitope. The C indicates the CGG linker containing the additional cysteine.

All other steps were performed by standard molecular biology protocols.

Sequence of the oligonucleotides:

5'LT[[•]]β:

5'-CTT GGT GCC GCA GGA TCA G-3' (SEQ ID NO:284)

3'LT[[•]]β:

5'-CAG ATG GCT GTC ACC CCA C-3' (SEQ ID NO:285)

5'LT[[•]]βlong-*Nhe*I:

5'-GCC CGC TAG CCT GCG GTG GTC AGG ATC AGG GAC GTC G-3' (SEQ ID NO:286)

5'LT[[•]]βshort-*Nhe*I:

5'-GCC CGC TAG CCT GCG GTG GTT CTC CAG CTG CGG ATT C -3' (SEQ ID NO:287)

3'LT[[•]]βstop-*Not*I

5'-CAA TGA CTG CGG CCG CTT ACC CCA CCA TCA CCG -3' (SEQ ID NO:288)

Expression and production of GST-EK-C-LT[[•]]β$_{49-306}$, GST-EK-C-LT[[•]]β$_{126-306}$, his-myc-EK-C-LT[[•]]β$_{49-306}$ and his-myc-EK-C-LT[[•]]β$_{126-306}$

The plasmids pCEP-SP-GST-EK-C-LT[[•]]β$_{49-306}$, pCEP-SP-GST-EK-C-LT[[•]]β 126-306, pCEP-SP-his-myc-EK-C-LT[[•]]β$_{49-306}$ and pCEP-SP-his-myc-EK-C-LT[[•]]β$_{126-306}$ were transfected into 293-EBNA cells (Invitrogen) for protein production as described in EXAMPLE 1. The resulting proteins were named GST-EK-C-LT[[•]]β$_{49-306}$, GST-EK-C-LT[[•]]β$_{126-306}$, his-myc-EK-C-LT[[•]]β$_{49-306}$ and his-myc-EK-C-LT[[•]]β$_{126-306}$.

The protein sequences of the LT[[•]]β fusion proteins were translated from the cDNA sequences:

GST-EK-C-LT[[•]]β$_{49-306}$: SEQ ID NO:289

GST-EK-C-LT[[•]]β$_{126-306}$: SEQ ID NO:290

his-myc-EK-C-LT[[•]]β$_{49-306}$: SEQ ID NO:291

his-myc-EK-C-LT[[•]]β$_{126-306}$: SEQ ID NO:292

The fusion proteins were analysed on 12% SDS-PAGE gels under reducing conditions. Gels were blotted onto nitrocellulose membranes. Membranes were blocked, incubated with a monoclonal mouse anti-myc antibody or with an anti-GST antibody. Blots were subsequently incubated with horse radish peroxidase-conjugated goat anti-mouse IgG or horse radish peroxidase-conjugated rabbit anti-goat IgG. The results are shown in FIG. 3. GST-EK-C-LT[[•]]β$_{49-306}$ and GST-EK-C-LT[[•]]β$_{126-306}$ could be detected with the anti-GST antibody at a molecular weight of 62 kDa and 48 kDa, respectively. his-myc-EK-C-

LT[[•]]β$_{49-306}$ and his-myc-EK-C-LT[[•]]β$_{126-306}$ could be detected with the anti-myc antibody at 40-56 kDa and 33-39 kDa, respectively.

FIG. 3A and FIG. 3B show the result of the expression of LT[[•]]β fusion proteins. LT[[•]]β fusion proteins were analysed on 12% SDS-PAGE gels under reducing conditions. Gels were blotted onto nitrocellulose membranes. Membranes were blocked, incubated either with a monoclonal mouse anti-myc antibody (dilution 1:2000) (FIG. 3A) or with an anti-GST antibody (dilution 1:2000) (FIG. 3B). Blots were subsequently incubated with horse radish peroxidase-conjugated goat anti-mouse IgG (dilutions 1:4000) (FIG. 3A) or horse radish peroxidase-conjugated rabbit anti-goat IgG (dilutions 1:4000) (FIG. 3B). A: Lane 1 and 2: his-myc-EK-C-LT[[•]]β$_{126-306}$. Lane 3 and 4: his-myc-EK-C-LT[[•]]β$_{49-306}$. B: Lane 1 and 2: GST-EK-C-LT[[•]]β$_{126-306}$. Lane 3 and 4: GST-EK-C-LT[[•]]β$_{49-306}$. Molecular weights of marker proteins are given on the left margin.

B. Purification of GST-EK-C-LT[[•]]β$_{49-306}$, GST-EK-C-LT[[•]]β$_{126-306}$, his-myc-EK-C-LT[[•]]β$_{49-306}$ and his-myc-EK-C-LT[[•]]β$_{126-306}$

GST-EK-C-LT[[•]]β$_{49-306}$ and GST-EK-C-LT[[•]]β$_{126-306}$ are purified on glutathione-sepharose column and his-myc-EK-C-LT[[•]]β$_{49-306}$ and his-myc-EK-C-LT[[•]]β$_{126-306}$ are purified on Ni-NTA sepharose column using standard purification protocols. The purified proteins are cleaved with enterokinase and analysed on a 16% SDS-PAGE gel under reducing conditions

C. Coupling of C-LT[[•]]β$_{49-306}$ and C-LT[[•]]β$_{126-306}$ to Qβ capsid protein

A solution of 120 μM Qβ capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25°C on a rocking shaker. The reaction solution is subsequently

dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed Qβ reaction mixture is then reacted with the C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ solution (end concentrations: 60 µM Qβ, 60 µM C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE.

D. Coupling of C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ to fr capsid protein

A solution of 120 µM fr capsid in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed fr capsid protein reaction mixture is then reacted with the C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ solution (end concentrations: 60 µM fr, 60 µM C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE under reducing conditions.

E. Coupling of C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ to HBcAg-Lys-2cys-Mut

A solution of 120 µM HBcAg-Lys-2cys-Mut capsid in 20 mM Hepes, 150 mM NaCl pH 7.2 is reacted for 30 minutes with a 25 fold molar excess of SMPH (Pierce), diluted from a stock solution in DMSO, at 25 °C on a rocking shaker. The reaction solution is subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 °C. The dialyzed HBcAg-Lys-2cys-Mut reaction mixture is then reacted with the C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ solution (end concentrations: 60 µM HBcAg-Lys-2cys-Mut, 60 µM C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE.

F. Coupling of C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ to Pili

A solution of 125 μM Type-1 pili of *E.coli* in 20 mM Hepes, pH 7.4, is reacted for 60 minutes with a 50-fold molar excess of cross-linker SMPH, diluted from a stock solution in DMSO (Pierce), at RT on a rocking shaker. The reaction mixture is desalted on a PD-10 column (Amersham-Pharmacia Biotech). The protein-containing fractions ~~eluating~~ eluting from the column are pooled, and the desalted derivatized pili protein is reacted with the C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$ solution (end concentrations: 60 μM pili, 60 μM C-LT[[•]]$\beta_{49\text{-}306}$ and C-LT[[•]]$\beta_{126\text{-}306}$) for four hours at 25 °C on a rocking shaker. Coupling products are analysed by SDS-PAGE under reducing conditions.

EXAMPLE 4

A. Introduction of cys-containing linkers, expression, purification and coupling of rat macrophage migration inhibitory factor MIF to Qβ

Rat macrophage migration inhibitory factor (rMIF) was recombinantly expressed with three different amino acid linkers C1, C2 and C3 fused at its C-terminus. Each of the linker contained one cysteine for coupling to VLP.

Construction of rMIF-C1, rMIF-C2, and rMIF-C3.

The MCS of pET22b(+) (Novagen, Inc.) was changed to GTTTAACTTT AAGAAGGAGATATACATATGGATCCGGCTAGCGCTCGAGGGTTTAAACGGCGGC CGCATGCACC (SEQ ID NO:363) by replacing the original sequence from the NdeI site to XhoI site with annealed oligos primerMCS-1F and primerMCS-1R (annealing in 15 mM TrisHCl pH 8 buffer). The resulting plasmid was termed pMod00, which had NdeI, BamHI, NheI, XhoI, PmeI and NotI restriction sites in its MCS. The annealed pair of oligos Bamhis6-

EK-Nhe-F and Bamhis6-EKNhe-R and the annealed pair of oligo1F-C-glycine-linker and oligo1R-C-glycine-linker were together ligated into BamHI-NotI digested pMod00 plasmid to get pModEC1, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C-terminal amino acid glycine linker containing one cysteine residue. The annealed pair of oligos Bamhis6-EK-Nhe-F and Bamhi6-EKNhe R together with the annealed pair of oligo1F-C-gamma1-linker and oligo1R-C-gamma1-linker were ligated into BamHI-NotI digested pMod00 plasmid to get pModEC2, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C-terminal 1 linker, derived from the hinge region of human immunoglobulin [[•]]γ1, containing one cysteine residue. The annealed pair of oligos Bamhis6-EK-Nhe-F and Bamhis6-EK-Nhe-R, the annealed pair of oligo1FA-C-gamma3-linker and oligo1RA-C-gamma3-linker, and the annealed pair of oligo1FB-C-gamma3-linker and oligo1RB-C-gamma3-linker were together ligated into BamHI-NotI digested pMod00 to get pModEC3, which had an N terminal hexahistidine tag, an enterokinase cleavage site and a C terminal [[•]]γ3 linker, containing one cysteine residue, derived from the hinge region of mouse immunoglobulin [[•]]γ3.

pBS-rMIF, which contains the rat MIF cDNA, was amplified by PCR with oligos rMIF-F and rMIF-Xho-R. rMIF-F had an internal NdeI site and rMIF-Xho-R had an internal XhoI site. The PCR product was digested with NdeI and XhoI and ligated into pModEC1, pModEC2 and pModEC3 digested with the same enzymes. Resulting plasmids were named pMod-rMIF-C1, pMod-rMIF-C2 and pMod-rMIF-C3, respectively.

For the PCR reaction, 15 pmol of each oligo and 1 ng of the template DNA was used in the 50 [[•]]μl reaction mixture (2 units of PFX polymerase, 0.3 mM dNTPs and 2 mM MgSO4). The temperature cycles were as follows: 94°C for 2 minutes, followed by 30 cycles of 94°C (30 seconds), 60°C (30 seconds), 68°C (30 seconds) and followed by 68°C for 2 minutes.

Please amend the specification from the first full paragraph on page 109 to the second full paragraph on page 110 as follows:

Coupling of rMIF-C1 to Q[[•]]β capsid protein

A solution of 1.48 ml of 6 mg/ml Q[[•]]β capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 30 minutes with 14.8 µl of a SMPH (Pierce) (from a 100 mM stock solution dissolved in DMSO) at 25°C. The reaction solution was subsequently dialyzed twice for 3 hours against 2 l of 20 mM Hepes, 150 mM NaCl, pH 7.0 at 4 [[>]]°C. A solution of 1.3 ml of 3.6 mg/ml rMIF-C1 protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 1 hour with 9.6 µl of a TCEP (Pierce) (from a 36 mM stock solution dissolved in H_2O) at 25°C. 130 µl of the derivatized and dialyzed Qβ was then reacted with 129 µl of reduced rMIF-C1 in 241 µl of 20 mM Hepes, 150 mM NaCl, pH 7.0 over night at 25°C.

Coupling of rMIF-C2 to Q[[•]]β capsid protein

A solution of 0.9 ml of 5.5 mg/ml Q[[•]]β capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 30 minutes with 9 µl of a SMPH (Pierce) (from a 100 mM stock solution dissolved in DMSO) at 25°C. The reaction solution was subsequently dialyzed twice for 2 hours against 2 l of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4 [[>]]°C. A solution of 850 µl of 5.80 mg/ml rMIF-C2 protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 1 hour with 8.5 µl of a TCEP (Pierce) (from a 36 mM stock solution dissolved in H_2O) at RT. 80 µl of the derivatized and dialyzed Q[[•]]β was then reacted with 85 µl of reduced rMIF-C2 in 335 µl of 20 mM Hepes, 150 mM NaCl, pH 7.2 over night at 25°C.

Coupling of rMIF-C3 to Q[[•]]β capsid protein

A solution of 1.48 ml of 6 mg/ml Q[[•]]β capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 30 minutes with 14.8 µl of a SMPH (Pierce) (from a 100 mM stock solution dissolved in DMSO) at 25°C. The reaction solution was subsequently dialyzed twice for 3 hours against 2 l of 20 mM Hepes, 150 mM NaCl, pH 7.0 at 4 [[>]]°C. A solution of 720 µl of 5.98 mg/ml rMIF-C3 protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 1 hour with 9.5 µl of a TCEP (Pierce) (from a 36 mM stock solution dissolved in H_2O) at 25°C. 130 µl of the derivatized and dialyzed Q[[•]]β was then reacted with 80 µl of reduced rMIF-C3 in 290 µl of 20 mM Hepes, 150 mM NaCl, pH 7.0 over night at 25°C.

All three coupled products were analysed on 16% SDS-PAGE gels under reducing conditions. Gels were either stained with Coomassie Brilliant Blue or blotted onto nitrocellulose membranes. Membranes were blocked, incubated with a polyclonal rabbit anti-Q[[b]]β antiserum (dilution 1:2000) or a purified rabbit anti-MIF antibody (Torrey Pines Biolabs, Inc.) (dilution 1:2000). Blots were subsequently incubated with horse radish peroxidase-conjugated goat anti-rabbit IgG (dilutions 1:2000). The results are shown in FIG 4A and FIG. 4B. Coupled products could be detected in the Coomassie-stained gels (FIG. 4A) and by both [[•]] anti-Qβ[[•]] antiserum and the anti-MIF antibody (FIG. 4B) clearly demonstrated the covalent coupling of all three rMIF variants to Qβ[[•]] capsid protein.

Please amend the first through fourth paragraphs on page 117 as follows:

A truncated form (aa 121-230) of the mouse prion protein (termed mPrPt) was recombinantly expressed with a GGGGCG (SEQ ID NO:413) amino acid linker fused at its C-terminus for coupling to VLPs and Pili. The protein was fused to the N-terminus of a human Fc-fragment for purification. An enterokinase (EK) cleavage-site was introduced behind the EK cleavage site to cleave the Fc- part of the fusion protein after purification.

Construction of mPrPt-EK-Fc*.

Mouse PrPt was amplified by PCR with the primer 5'PrP-*BamH*I and 3'PrP-*Nhe*I using the plasmid pBPCMVPrP-Fc as a template. pBPCMVPrP-Fc contained the wild-type sequence of the mouse prion protein. 5'PrP-*BamH*I had an internal *BamH*I site and contained an ATG and 3'PrP-*Nhe*I had an internal *Nhe*I site.

For the PCR reaction, 0.5 μg of each primer and 200 ng of the template DNA was used in the 50 μl reaction mixture (1 unit of PFX Platinum polymerase, 0.3 mM dNTPs and 2 mM MgSO4). The temperature cycles were as follows: 94□C for 2 minutes, followed by 5 cycles of 94[[□]]°C (15 seconds), 50[[□]]°C (30 seconds), 68[[□]]°C (45 seconds), followed by 20 cycles of 94°C (15 seconds), 64°C (30 seconds), 68°C (45 seconds) and followed by 68[[□]]°C for 10 minutes.

The PCR product was digested with *BamH*I and *Nhe*I and inserted into pCEP-SP-EK-Fc* containing the GGGGCG (SEQ ID NO:413) linker sequence at the 5'end of the EK cleavage sequence. The resulting plasmid was named pCEP-SP-mPrPt-EK-Fc*.

Please amend the second and third full paragraphs on page 118 as follows:

The protein sequence of the mPrPt-EK-Fc* is identified in SEQ ID NO:323. mPrPt after cleavage has the sequence as identified in SEQ ID NO:324 with the GGGGCG (SEQ ID NO:413) linker at its C-terminus.

The purified fusion protein mPrPt-EK-Fc* was cleaved with enterokinase and analysed on a 16% SDS-PAGE gel under reducing conditions before and after enterokinase cleavage. The gel was stained with Coomassie Brilliant Blue. The result is shown in FIG. 7. Molecular weights of marker proteins are given on the left margin of the gel in the figure. The mPrPt-EK-Fc* fusion protein could be detected as a 50 kDa band. The cleaved mPrPt

protein containing the GGGGCG (SEQ ID NO:413) amino acid linker fused to its C-terminus could be detected as a broad band between 18 and 25 kDa. The identity of mPrPt was confirmed by western blotting (data not shown). Thus, mPrPt with a C-terminal amino acid linker containing a cysteine residue, could be expressed and purified to be used for coupling to VLPs and Pili.

Please amend the first through fourth paragraphs on page 120 as follows:

The following prion peptides were chemically synthesized: CSAMSRPMIHFGNDWEDRYYRENMYR ("cprplong"; SEQ ID NO:364) and CGNDWEDRYYRENMYR ("cprpshort"; SEQ ID NO:365), which comprise an added N-terminal cysteine residue for coupling to VLPs and Pili, and used for chemical coupling to Qβ as described in the following.

A solution of 5 ml of 140 μM Qβ capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 108 μl of a 65 mM solution of SMPH (Pierce) in H_2O at 25°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 5 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4°C. 100 μl of the dialyzed reaction mixture was then reacted either with 1.35 μl of a 2 mM stock solution (in DMSO) of the peptide cprpshort (1:2 peptide/Q[[•]]β capsid protein ratio) or with 2.7 μl of the same stock solution (1:1 peptide/Q[[•]]β ratio). 1 μl of a 10 mM stock solution (in DMSO) of the peptide cprplong was reacted with 100 μ1 of the dialyzed reaction mixture. The coupling reactions were performed over night at 15 °C in a water bath. The reaction mixtures were subsequently dialyzed 24 h against 2x 5 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4°C.

The coupled products were centrifuged and supernatants and pellets were analysed on 16% SDS-PAGE gels under reducing conditions. Gels were stained with Coomassie Brilliant Blue. The results are shown in FIG. 16. Molecular weights of marker proteins are given on

the left margin of the gel in the figure. The bands at a molecular weight between 16.5 and 25 kDa clearly demonstrated the covalent coupling of the peptides cprpshort and cprplong to Q[[•]]β capsid protein.

The samples loaded on the gel of FIG. 16 A were the following:

Lane 1: purified Q[[•]]β capsid protein. Lane 2: derivatized Qβ capsid protein before coupling. Lanes 3-6: Qβ capsid protein-cprpshort couplings with a 1:2 peptide/Q[[•]]β ratio (lanes 3 and 4) and 1:1 peptide/Q[[•]]β ratio (lanes 5 and 6). Soluble fractions (lanes 3 and 5) and insoluble fractions (lanes 4 and 6) are shown.

Please amend the first full paragraph on page 123 as follows:

The cDNA used for cloning IL-13 with an N-terminal GST originated from the cDNA of TH2 ~~actiated~~ activated T-cells as described above (a.). IL-13 was amplified from this cDNA using the primers Nhelink1IL13-F and IL13StopXhoNot-R. The PCR product was digested with NheI and XhoI and ligated in the pCEP-SP-GST-EK vector previously digested with NheI/XhoI. The plasmid which could be isolated from the ligation (pCEP-SP-GST-IL13) was used to transfect HEK-293T cells. The resulting IL 13 construct encoded by this plasmid had the amino acid sequence LACGGGGG (SEQ ID NO:417) fused at the N-terminus of the IL-13 mature sequence. This sequence comprises the amino acid linker sequence ACGGGGG (SEQ ID NO:418) flanked by an additional amino acid introduced during the cloning procedure. The culture supernatant of the cells transfected with pCEP-SP-GST-IL13 contained the fusion protein GST-IL13 which could be purified by Glutathione affinity chromatography according to standard protocols. Mature IL-13 fused at its N-terminus with aforementioned amino acid sequence is released upon cleavage of the fusion

protein with enterokinase, leading to a protein called hereinafter "mouse C-IL-13-S" and having a sequence of SEQ ID NO:329.

Please amend the second full paragraph on page 126 as follows:

The template described under (A) (ATCC clone 37562) was used for the cloning of the following construct. The plasmid pMODB1-IL5 (a pET derivative) was digested with BamHI/XhoI to yield a small ~~fragement~~ fragment encoding IL5 fused to an N terminal amino acid linker containing a cysteine. This fragment was ligated in the vector pCEP-SP-XA-Fc*(ΔXho) which had previously been digested with BamHI and XhoI. The ligation was electroporated into *E.coli* strain TG1 and plasmid DNA of resulting clone pCEP-SP-IL5-Fc.2, whose sequence had been confirmed by DNA sequencing, was used to transfect HEK-293T cells. The resulting IL-5 construct encoded by this plasmid had the amino acid sequence ADPGCGGGGGLA (SEQ ID NO:419) fused at the N-terminus of the IL-5 mature sequence. This sequence comprises the amino acid linker sequence GCGGGGG (SEQ ID NO:420) containing a cysteine and flanked by additional amino acids introduced during the cloning procedure. The IL-5 protein released by cleavage of the fusion protein with Factor-Xa is named hereinafter "mouse C-IL-5-F" (SEQ ID NO:333).

Please amend the second full paragraph on page 127 as follows:

IL-5 (ATCC 37562) was amplified with the primers Nhe-link1-IL13-F and IL5StopXho-R. After digestion with NheI and XhoI the insert was ligated into pCEP-SP-GST-EK which had been previously digested with NheI and XhoI. The resulting plasmid pCEP-SP-GST-IL5 was sequenced and used for transfection of HEK-293T cells. The resulting IL-5 construct encoded by this plasmid had the amino acid sequence LACGGGGG (SEQ ID NO:417) fused at the N-terminus of the IL-5 mature sequence. This sequence

comprises the amino acid linker sequence ACGGGGG (SEQ ID NO:418) containing a cysteine residue and flanked by additional amino acids introduced during the cloning procedure. The protein released by cleavage with enterokinase was named hereinafter "mouse C-IL-5-S" (SEQ ID NO:334). The purification of the resulting protein was performed by affinity chromatography on Glutathione affinity resin.

Please amend the specification from the second paragraph on page 129 to the end of page 132 as follows:

Expression of recombinant mVEGFR-2(2-3) in eukaryotic cells

Recombinant mVEGFR-2(2-3) was expressed in EBNA 293 cells using the pCEP-SP-EK-Fc* vector. The pCEP-SP-EK-Fc* vector has a BamHI and an Nhe1 sites, encodes an amino acid linker containing one cysteine residue, an enterokinase cleavage site, and C-terminally a human Fc region. The mVEGFR-2(2-3) was amplified by PCR with the primer pair BamH1-FLK1-F and Nhe1-FLK1-B from a mouse 7-day embryo cDNA (Marathon-Ready cDNA, Clontech). For the PCR reaction, 10 pmol of each oligo and 0.5 ng of the cDNA (mouse 7-day embryo cDNA Marathon-Ready cDNA, Clontech) was used in the 50 [[•]]µl reaction mixture (1 [[•]]µl of Advantage 2 polymerase mix (50x), 0.2 mM dNTPs and 5 [[•]]µl 10x cDNA PCR reaction buffer). The temperature cycles were as follows: 5 cycles a 94[[•]]°C for 1 minute, 94[[•]]°C for 30 seconds, 54[[•]]°C for 30 seconds, 72[[•]]°C for 1 minute followed by 5 cycles of 94[[•]]°C (30 seconds), 54[[•]]°C (30 seconds), 70[[•]]°C (1 minute) and followed by 30 cycles 94[[•]]°C (20 seconds), 54[[•]]°C (30 seconds) and 68[[•]]°C (1 minute). The PCR product was digested with BamH1 and Nhe1 and inserted into the pCEP-SP-EK-Fc* vector digested with the same enzymes. Resulting plasmid was named mVEGFR-2(2-3)-pCep-EK-Fc. All other steps were performed by standard molecular biology protocols.

Oligos:

1. Primer BamH1-FLK1-F

   5'-CGCGGATCCATTCATCGCCTCTGTC-3' (SEQ ID NO:343)

2. Primer Nhe1-FLK1-B

   5'-CTAGCTAGCTTTGTGTGAACTCGGAC-3' (SEQ ID NO:344)

Transfection and expression of recombinant mVEGFR-2(2-3)

EBNA 293 cells were transfected with the mVEGFR-2(2-3)-pCep-Ek-Fc construct described above and serum free supernatant of cells was harvested for purification as described in EXAMPLE 1.

Purification of recombinant mVEGFR-2(2-3)

Protein A purification of the expressed Fc-EK-mVEGFR-2(2-3) proteins was performed as described in EXAMPLE 1. Subsequently, after binding of the fusion protein to Protein A, mVEGFR-2(2-3) was cleaved from the Fc portion bound to protein A using enterokinase (EnterokinaseMax, Invitrogen). Digestion was conducted over night at 37[[•]]°C (2,5 units enterokinase/100 [[□]]µl Protein A beads with bound fusion protein). The released VEGFR-2(2-3) was separated from the Fc-portion still bound to protein A beads by short centrifugation in chromatography columns (Micro Bio Spin, Biorad). In order to remove the enterokinase the flow through was treated with enterokinase away (Invitrogen) according to the instructions of the manufacturer.

EXAMPLE 12

Coupling of murine VEGFR-2 peptide to Qß capsid protein , HbcAg-lys-2cys-Mut and Pili and immunization of mice with VLP-peptide and Pili-peptide vaccines

A. Coupling of murine VEGFR-2 peptides to VLPs and pili

The following peptide[[s]] was chemically synthesized (by Eurogentec, Belgium): murine VEGFR-2 peptide CTARTELNVGLDFTWHSPPSKSHHKK (SEQ ID NO:366) and used for chemical coupling to Pili as described below.

Coupling of murine VEGFR-2 peptides to pili: A solution of 1400 µl of 1 mg/ml pili protein in 20 mM Hepes, pH 7.4, was reacted for 60 minutes with 85µl of a 100 mM Sulfo-MBS (Pierce) solution in (H_2O) at RT on a rocking shaker. The reaction mixture was desalted on a PD-10 column (Amersham-Pharmacia Biotech), The protein-containing fractions eluting from the column were pooled (containing approximately 1,4 mg protein) and reacted with a 2.5-fold molar excess (final volume) of murine VEGFR-2 peptide respectively. For example, to 200 µl eluate containing approximately 0[[,]].2 mg derivatized pili, 2.4 µl of a 10 mM peptide solution (in DMSO) were added. The mixture was incubated for four hours at 25 [[>]]°C on a rocking shaker and subsequently dialyzed against 2 liters of 20 mM Hepes, pH 7.2 overnight at 4[[>]]°C. Coupling results were analyzed by SDS-PAGE under reducing conditions and are shown in FIG. 18 A. Supernatant (S) and pellet (P) of each sample were loaded on the gel, as well pili and pili derivatized with Sulfo-MBS cross-linker (Pierce).The samples loaded on the gel of FIG. 18 A were the following:

Lane 1: Marker proteins; lane 2-5: coupled samples (Pili murine: Pili coupled to murine peptide; Pili human: Pili coupled to human peptide); lane 6: pili derivatized with

Sulfo-MBS cross-linker; lane 7-9: three fractions of the eluate of the PD-10 column. Fraction 2 is the peak fraction, fraction 1 and 3 are fractions taken at the border of the peak. Coupling bands were clearly visible on the gel, demonstrating the successful coupling of murine VEGFR-2 to pili.

Coupling of murine VEGFR-2 peptide to Q[[□]]βcapsid protein: A solution of 1 ml of 1 mg/ml Qβ capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.4 was reacted for 45 minutes with 20 µl of 100 mM Sulfo-MBS (Pierce) solution in (H_2O) at RT on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, pH 7.4 at 4[[>]]°C. 1000 µl of the dialyzed reaction mixture was then reacted with 12 µl of a 10 mM peptide solution (in DMSO) for four hours at 25[[o]]°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x2 hours against 2 liters of 20 mM Hepes, pH 7.4 at 4[[>]]°C. Coupling results were analyzed by SDS-PAGE under reducing conditions and are shown in FIG. 18 B. Supernatant (S) of each sample was loaded on the gel, as well as Qβ capsid protein and Qβ capsid protein derivatized with Sulfo-MBS cross-linker. Coupling was done in duplicate. The following samples were loaded on the gel:

Lane 1: Marker proteins; lane 2, 5: Qβ capsid protein; lane 3, 6 Qβ capsid protein derivatized with Sulfo-MBS; lane 4, 7: Qβ capsid protein coupled to murine VEGFR-2 peptide. Coupling bands were clearly visible on the gel, demonstrating the successful coupling of murine VEGFR-2 to Qβ capsid protein.

Coupling of murine VEGFR-2 peptide to HbcAg-lys-2cys-Mut: A solution of 3 ml of 0.9 mg/ml cys-free HbcAg capsid protein (EXAMPLE 31) in PBS, pH 7.4 was reacted for 45 minutes with 37[[,]]5 µl of a 100 mM Sulfo-MBS (Pierce) solution in (H_2O) at RT on a rocking shaker. The reaction solution was subsequently dialyzed overnight against 2 L of 20

mM Hepes, pH 7.4. After buffer exchange the reaction solution was dialyzed for another 2 hours against the same buffer. The dialyzed reaction mixture was then reacted with 3 µl of a 10 mM peptide solution (in DMSO) for 4 hours at 25[[>]]°C on a rocking shaker. The reaction mixture was subsequently dialyzed against 2 liters of 20 mM Hepes, pH 7.4 overnight at 4[[>]]°C followed by buffer exchange and another 2 hours of dialysis against the same buffer. Coupling results were analyzed by SDS-PAGE under reducing conditions and are shown in FIG. 18 C. The supernatant (S) of each sample was loaded on the gel, as well as HbcAg-lys-2cys-Mut protein and HbcAg-lys-2cys-Mut protein derivatized with Sulfo-MBS cross-linker. Coupling was done in duplicate. Coupling reactions were conducted in a 2.5 fold and 10 fold molar excess of peptide. The following samples were loaded on the gel:

Please amend the specification from first full paragraph on page 135 to the second full paragraph on page 136 as follows:

The following Aβ peptide was chemically synthesized (DAEFRHDSGYEVHHQGGC (SEQ ID NO:367)), a peptide which comprises the amino acid sequence from residue 1-15 of human Aβ, fused at its C-terminus to the sequence GGC for coupling to VLPs and Pili.

A. a.) Coupling of Aβ 1-15 peptide to HBc-Ag-lys-2cys-Mut using the cross-linker SMPH.

A solution of 833.3 µl of 1.2 mg/ml HBc-Ag-lys-2cys-Mut protein in 20 mM Hepes 150 mM NaCl pH 7.4 was reacted for 30 minutes with 17 µl of a solution of 65 mM SMPH (Pierce) in H_2O, at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C in a dialysis tubing with Molecular Weight cutoff 10000 Da. 833.3 µl of the dialyzed reaction mixture was then reacted with 7.1 µl of a 50 mM peptide stock solution

(peptide stock solution in DMSO) for two hours at 15°C on a rocking shaker. The reaction mixture was subsequently dialyzed overnight against 1 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. The sample was then frozen in aliquots in liquid Nitrogen and stored at –80°C until immunization of the mice.

b) Coupling of Aβ 1-15 peptide to fr capsid protein using the cross-linker SMPH..

A solution of 500 µl of 2 mg/ml fr capsid protein in 20 mM Hepes 150 mM NaCl pH 7.4 was reacted for 30 minutes with 23 µl of a solution of 65 mM SMPH (Pierce) in H_2O, at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 1 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C in a dialysis tubing with Molecular Weight cutoff 10000 Da. 500µl of the dialyzed reaction mixture was then reacted with 5.7 µl of a 50 mM peptide stock solution (peptide stock solution in DMSO) for two hours at 15°C on a rocking shaker. The reaction mixture was subsequently dialyzed overnight against 1 liter of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. The sample was then frozen in aliquots in liquid Nitrogen and stored at –80°C until immunization of the mice. Samples of the coupling reaction were analyzed by SDS-PAGE under reducing conditions.

Please amend the second through fourth paragraphs on page 138 as follows:

The following Aβ peptides were chemically synthesized: DAEFRHDSGYEVHHQGGC ("Aβ 1-15"; SEQ ID NO:367), a peptide which comprises the amino acid sequence from residue 1-15 of human Aβ fused at its C-terminus to the sequence GGC for coupling to Pili and VLPs, DAEFRHDSGYEVHHQKLVFFAEDVGSNGGC ("Aβ 1-27"; SEQ ID NO:368) a peptide which comprises the amino acid sequence from residue 1-

27 of human Aβ fused at its C-terminus to the sequence GGC for coupling to Pili and VLPs, and CGHGNKSGLMVGGVVIA ("Aβ 33-42"; SEQ ID NO:369) a peptide which comprises the amino acid sequence from residue 33-42 of Aβ, fused at its N-terminus to the sequence CGHGNKS (SEQ ID NO:405) for coupling to Pili and VLPs. All three peptides were used for chemical coupling to Pili as described in the following.

A solution of 2 ml of 2 mg/ml Pili in 20 mM Hepes 150 mM NaCl pH 7.4 was reacted for 45 minutes with 468 µl of a solution of 33.3 mM SMPH (Pierce) in H_2O, at 25[[>]]°C on a rocking shaker. The reaction solution was loaded on a PD10 column (Pharmacia) and eluted with 6 X 500 µl of 20 mM Hepes 150mM NaCl pH 7.4. Fractions were analyzed by dotting on a Nitrocellulose (Schleicher & Schuell) and stained with Amidoblack. Fractions 3 – 6 were pooled. The samples were then frozen in aliquots in liquid Nitrogen and stored at –80°C until coupling.

200 µl of the thawed desalted reaction mixture was then mixed with 200 µl DMSO and 2.5 µl of each of the corresponding 50 mM peptide stock solutions in DMSO, for 3.5 hours at RT on a rocking shaker. 400 µl of the reaction mixture was subsequently dialyzed three times for one hour against 1 liter of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C in a dialysis tubing with Molecular Weight cutoff 10000 Da. The samples were then frozen in aliquots in liquid Nitrogen and stored at –80°C

Please amend the second full paragraph on page 139 as follows:

Three different Aß peptides (Aß 1-27-Gly-Gly-Cys-NH2 (SEQ ID NO:421); H-Cys-Gly-His-Gly-Asn-Lys-Ser-Aß 33-42 (SEQ ID NO:422); Aß 1-15-Gly-Gly-Cys-NH2 (SEQ ID NO:422)) were coupled to Qβcapsid protein. The resulting vaccines were termed "Qb-Ab 1-15", "Qb-Ab 1-27" and "Qb-Ab 33-42". 8 months old female APP23 mice which carry a human APP transgene (Sturchler-Pierrat *et al., Proc.Natl. Acad.Sci. USA 94*: 13287-13292

(1997)) were used for vaccination. The mice were injected subcutaneously with 25 μg vaccine diluted in sterile PBS and 14 days later boosted with the same amount of vaccine. Mice were bled from the tail vein before the start of immunization and 7 days after the booster injection. The sera were analyzed by ELISA.

Please amend the specification from the first full paragraph on page 143 to the second full paragraph on page 146 as follows:

The plasmid pQβ10 (Kozlovska, TM, et al., Gene 137:133-137) was used as an initial plasmid for the construction of pQβ-240. The mutation Lys13→Arg was created by inverse PCR. The inverse primers were designed in inverted tail-to-tail directions:

5'-GGTAACATCGGTCGAGATGGAAAACAAACTCTGGTCC-3' (SEQ ID NO: 370) and 5'-GGACCAGAGTTTGTTTTCCATCTCGACCGATGTTACC-3' (SEQ ID NO: 371).

The products of the first PCR were used as templates for the second PCR reaction, in which an upstream primer

5'-AGCTCGCCCGGGGATCCTCTAG-3' (SEQ ID NO:372) and a downstream primer

5'-CGATGCATTTCATCCTTAGTTATCAATACGCTGGGTTCAG-3' (SEQ ID NO:373) were used. The product of the second PCR was digested with XbaI and Mph1103I and cloned into the pQβ10 expression vector, which was cleaved by the same restriction enzymes. The PCR reactions were performed with PCR kit reagents and according to producer protocol (MBI Fermentas, Vilnius, Lithuania).

Sequencing using the direct label incorporation method verified the desired mutations. *E.coli* cells harbouring pQβ-240 supported efficient synthesis of 14-kD protein co migrating upon PAGE with control Qβ coat protein isolated from Qβ phage particles.

Resulting amino acid sequence: (SEQ ID NO:255)

AKLETVTLGNIGRDGKQTLVLNPRGVNPTNGVASLSQAGAVP

ALEKRVTVSVSQPSRNRKNYKVQVKIQNPTACTANGSCDPSVTRQ

KYADVTFSFTQYSTDEERAFVRTELAALLASPLLIDAIDQLNPAY

Construction of pQβ-243

The plasmid pQβ10 was used as an initial plasmid for the construction of pQβ-243.The mutation Asn10→Lys was created by inverse PCR. The inverse primers were designed in inverted tail-to-tail directions:

5'-GGCAAAATTAGAGACTGTTACTTTAGGTAAGATCGG-3' (SEQ ID NO:374) and

5'-CCGATCTTACCTAAAGTAACAGTCTCTAATTTTGCC-3' (SEQ ID NO:375).

The products of the first PCR were used as templates for the second PCR reaction, in which an upstream primer

5'-AGCTCGCCCGGGGATCCTCTAG-3' (SEQ ID NO:372) and a downstream primer

5'-CGATGCATTTCATCCTTAGTTATCAATACGCTGGGTTCAG-3' (SEQ ID NO:373) were used. The product of the second PCR was digested with ***XbaI*** and ***Mph1103I*** and cloned into the pQβ10 expression vector, which was cleaved by the same restriction enzymes. The PCR reactions were performed with PCR kit reagents and according to producer protocol (MBI Fermentas, Vilnius, Lithuania).

Sequencing using the direct label incorporation method verified the desired mutations. *E.coli* cells harbouring pQβ-243 supported efficient synthesis of 14-kD protein co migrating upon PAGE with control Qβ coat protein isolated from Qβ phage particles.

Resulting amino acid sequence: (SEQ ID NO:256)

AKLETVTLGKIGKDGKQTLVLNPRGVNPTNGVASLSQAGAVP
ALEKRVTVSVSQPSRNRKNYKVQVKIQNPTACTANGSCDPSVTRQ
KYADVTFSFTQYSTDEERAFVRTELAALLASPLLIDAIDQLNPAY

Construction of pQ[[()]β-250

The plasmid pQβ-240 was used as an initial plasmid for the construction of pQβ-250. The mutation Lys2→Arg was created by site-directed mutagenesis. An upstream primer 5'-GGCCATGGCACGACTCGAGACTGTTACTTTAGG-3' (SEQ ID NO:376) and a downstream primer 5'-GATTTAGGTGACACTATAG-3' (SEQ ID NO:377) were used for the synthesis of the mutant PCR-fragment, which was introduced into the pQβ-185 expression vector at the unique restriction sites ***NcoI*** and ***HindIII***. The PCR reactions were performed with PCR kit reagents and according to producer protocol (MBI Fermentas, Vilnius, Lithuania).

Sequencing using the direct label incorporation method verified the desired mutations. *E.coli* cells harbouring pQβ-250 supported efficient synthesis of 14-kD protein co migrating upon PAGE with control Qβ coat protein isolated from Qβ phage particles.

Resulting amino acid sequence: (SEQ ID NO:257)

ARLETVTLGNIGRDGKQTLVLNPRGVNPTNGVASLSQAGAVP
ALEKRVTVSVSQPSRNRKNYKVQVKIQNPTACTANGSCDPSVTRQ
KYADVTFSFTQYSTDEERAFVRTELAALLASPLLIDAIDQLNPAY

Construction of pQβ[[Q]]-251

The plasmid pQβ10 was used as an initial plasmid for the construction of pQβ-251. The mutation Lys16→Arg was created by inverse PCR. The inverse primers were designed in inverted tail-to-tail directions:

5'-GATGGACGTCAAACTCTGGTCCTCAATCCGCGTGGGG -3' (SEQ ID NO:378) and 5'-CCCCACGCGGATTGAGGACCAGAGTTTGACGTCCATC -3' (SEQ ID NO:379).

The products of the first PCR were used as templates for the second PCR reaction, in which an upstream primer

5'-AGCTCGCCCGGGGATCCTCTAG-3' (SEQ ID NO:372) and a downstream primer

5'-CGATGCATTTCATCCTTAGTTATCAATACGCTGGGTTCAG-3' (SEQ ID NO:373) were used. The product of the second PCR was digested with ***XbaI*** and ***Mph1103I*** and cloned into the pQβ10 expression vector, which was cleaved by the same restriction enzymes. The PCR reactions were performed with PCR kit reagents and according to producer protocol (MBI Fermentas, Vilnius, Lithuania).

Sequencing using the direct label incorporation method verified the desired mutations. *E.coli* cells harbouring pQβ-251 supported efficient synthesis of 14-kD protein co migrating upon PAGE with control Qβ coat protein isolated from Qβ phage particles. The resulting amino acid sequence encoded by this construct is shown in SEQ. ID NO:259.

Construction of pQβ-259

The plasmid pQβ-251 was used as an initial plasmid for the construction of pQβ-259. The mutation Lys2→Arg was created by site-directed mutagenesis. An upstream primer 5'-GGCCATGGCACGACTCGAGACTGTTACTTTAGG-3' (SEQ ID NO:376) and a

downstream primer 5'-GATTTAGGTGACACTATAG-3' (SEQ ID NO:377) were used for the synthesis of the mutant PCR-fragment, which was introduced into the pQβ-185 expression vector at the unique restriction sites ***NcoI*** and ***HindIII***. The PCR reactions were performed with PCR kit reagents and according to producer protocol (MBI Fermentas, Vilnius, Lithuania).

Please amend the first and second paragraphs on page 147 as follows:

Transform E.coli JM109 with Q-beta expression plasmids. Inoculate 5 ml of LB liquid medium with 20 [[•]]μg/ml ampicillin with clones transformed with Q-beta expression plasmids. Incubate at 37 °C for 16-24 h without shaking.

Inoculate 100-300 ml of LB medium, containing 20 [[•]]μg/ml, 1:100 with the prepared inoculum. Incubate at 37 °C overnight without shaking. Inoculate M9 + 1 % Casamino acids + 0.2 % glucose medium in flasks with the prepared inoculum 1:50, incubate at 37 °C overnight under shaking.

Please amend the third full paragraph on page 154 as follows:

A solution of 4.0 mg/ml Qβ capsid protein in 20 mM Hepes, 150 mM NaCl pH 7.2 was reacted for 30 minutes with 10 fold molar excess SMPH (Pierce) (from a 100 mM stock solution dissolved in DMSO) at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 l of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. The VAE051 solution (2.4 mg/ml) was reducted with an equimolar concentration of TCEP for 60 min at 25[[>]]°C.

Please amend the first and second paragraphs on page 156 as follows:

The Derp 1,2 peptide, to which a cysteine was added N-terminally for coupling, was chemically synthesized and had the following sequence: H2N-CQIYPPNANKIREALAQTHSA-COOH (SEQ ID NO:385). This peptide was used for chemical coupling to wt Qβ capsid protein and as described in the following.

D. Coupling of Flag peptide to Qβ capsid protein

Qβ capsid protein in 20 mM Hepes, 150 mM NaCl, pH 7.2, at a concentration of 2 mg/ml, was reacted with a 5- or 20- fold excess of the cross-linker SMPH (Pierce) for 30 min. at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. The dialyzed reaction mixture was then reacted with a 5-fold excess of Derp 1,2 peptide for two hours at 25 °C on a rocking shaker.

Please amend the first and second paragraphs on page 157 as follows:

The c/e1 epitope (residues 72 to 88) of HBcAg is located in the tip region on the surface of the Hepatitis B virus capsid (HBcAg). A part of this region (Proline 79 and Alanine 80) was genetically replaced by the peptide Gly-Gly-Lys-Gly-Gly (HBcAg-Lys construct; SEQ ID NO:406). The introduced Lysine residue contains a reactive amino group in its side chain that can be used for intermolecular chemical crosslinking of HBcAg particles with any antigen containing a free cysteine group.

HBcAg-Lys DNA, having the amino acid sequence shown in SEQ ID NO:158, was generated by PCRs: The two fragments encoding HBcAg fragments (amino acid residues 1 to 78 and 81 to 149) were amplified separately by PCR. The primers used for these PCRs also introduced a DNA sequence encoding the Gly-Gly-Lys-Gly-Gly (SEQ ID NO:406) peptide.

The HBcAg (1 to 78) fragment was amplified from pEco63 using primers EcoRIHBcAg(s) and Lys-HBcAg(as). The HBcAg (81 to 149) fragment was amplified from pEco63 using primers Lys-HBcAg(s) and HBcAg(1-149)Hind(as). Primers Lys-HBcAg(as) and Lys-HBcAg(s) introduced complementary DNA sequences at the ends of the two PCR products allowing fusion of the two PCR products in a subsequent assembly PCR. The assembled fragments were amplified by PCR using primers EcoRIHBcAg(s) and HbcAg(1-149)Hind(as).

Please amend the third paragraph on page 185 as follows:

The following angiotensin peptides were chemically synthesized: CGGDRVYIHPF ("Angio I"; SEQ ID NO:380), CGGDRVYIHPFHL ("Angio II"; SEQ ID NO:381), DRVYIHPFHLGGC ("Angio III"; SEQ ID NO:382), CDRVYIHPFHL ("Angio IV"; SEQ ID NO:383) and used for chemical coupling to Qβ as described in the following.

Please amend the first paragraph on page 187 as follows:

The following angiotensin peptides were chemically synthesized: CGGDRVYIHPF ("Angio I"; SEQ ID NO:380), CGGDRVYIHPFHL ("Angio II"; SEQ ID NO:381), DRVYIHPFHLGGC ("Angio III"; SEQ ID NO:382), CDRVYIHPFHL ("Angio IV"; SEQ ID NO:383) and are used for chemical coupling to HBcAg-149-lys-2cys-Mut, i.e. cys-free HBcAg.

Please amend the third paragraph on page 187 as follows:

The following angiotensin peptides were chemically synthesized: CGGDRVYIHPF ("Angio I"; SEQ ID NO:380), CGGDRVYIHPFHL ("Angio II"; SEQ ID NO:381),

DRVYIHPFHLGGC ("Angio III"; SEQ ID NO:382), CDRVYIHPFHL ("Angio IV"; SEQ ID NO:383) and are used for chemical coupling to Type-1 pili of *E.coli*.

Please amend the first paragraph on page 188 as follows:

The following peptides derived from the house dust mite allergen Der p I were chemically synthesized: CGNQSLDLAEQELVDCASQHGCH ("Der p I p52"; aa 52-72, with an additional cysteine-glycine linker at the N terminus; SEQ ID NO:384), CQIYPPNANKIREALAQTHSA ("Der p 1 p117"; aa 117-137; SEQ ID NO:385). These peptides were used for chemical coupling to Qβ as described below.

Please amend the specification from the fourth paragraph on page 189 to the second paragraph on page 190 as follows:

The following peptides derived from the house dust mite allergen Der p 1 were chemically synthesized: Der p I p52 (aa 52-72, with an additional cysteine-glycine linker at the N terminus): CGNQSLDLAEQELVDCASQHGCH (SEQ ID NO:384), Der p I p117 (aa 117-137): CQIYPPNANKIREALAQTHSA (SEQ ID NO:385). These peptides are used for chemical coupling to HBcAg-149-lys-2cys-Mut, i.e. cys-free HBcAg.

A solution of 1.25 ml of 0.8 mg/ml HBcAg-149-lys-2cys-Mut capsid protein (Example 31) in PBS, pH 7.4 is reacted for 30 minutes with 93 µl of a solution of 13 mg/ml Sulfo-MBS (Pierce) in H_2O at 25[[»]]°C on a rocking shaker. The reaction solution is subsequently dialyzed overnight against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4. After buffer exchange the reaction solution is dialyzed for another 2 hours. The dialyzed reaction mixture is then reacted with 1.8 µl of a 100 mM peptide stock solution (in DMSO) for 2 hours at 25°C on a rocking shaker. The reaction mixture is subsequently dialyzed against 2

liters of 20 mM Hepes, 150 mM NaCl, ph 7.4 overnight at 4°C followed by buffer exchange and another 2 hours of dialysis.

The following peptides derived from the house dust mite allergen Der p I were chemically synthesized: Der p I p52 (aa 52-72, with an additional cysteine-glycine linker at the N terminus) and CGNQSLDLAEQELVDCASQHGCH (SEQ ID NO:384), Der p I p117 (aa 117-137): CQIYPPNANKIREALAQTHSA (SEQ ID NO:385). These peptides are used for chemical coupling to Type-1 pili of *E.coli*.

Please amend the third paragraph on page 190 as follows:

The human VEGFR II peptide with the sequence CTARTELNVGIDFNWEYPSSKHQHKK (SEQ ID NO:351) was chemically synthesized and used for chemical coupling to Type-1 pili of *E.coli*.

Please amend the first full paragraph on page 192 as follows:

The human VEGFR II peptide with the sequence CTARTELNVGIDFNWEYPSSKHQHKK (SEQ ID NO:351) was chemically synthesized and is used for chemical coupling to Qβ capsid protein.

Please amend the first paragraph on page 194 as follows:

The human VEGFR II peptide with the sequence CTARTELNVGIDFNWEYPSSKHQHKK (SEQ ID NO:351) was chemically synthesized and is used for chemical coupling to HBcAg-149-lys-2cys-Mut capsid protein.

Please amend the specification from the third paragraph on page 194 to the end of page 195 as follows:

Hepatitis core Antigen (HBcAg) 1-183 was modified as described in Example 23. A part of the c/e1 epitope (residues 72 to 88) region (Proline 79 and Alanine 80) was genetically replaced by the peptide Gly-Gly-Lys-Gly-Gly (HBcAg1-183Lys construct; SEQ ID NO:406). The introduced Lysine residue contains a reactive amino group in its side chain that can be used for intermolecular chemical crosslinking of HBcAg particles with any antigen containing a free cysteine group. PCR methods essentially as described in Example 1 and conventional cloning techniques were used to prepare the HBcAg1-183Lys gene.

The Gly-Gly-Lys-Gly-Gly (SEQ ID NO:406) sequence was inserted by amplifying two separate fragments of the HBcAg gene from pEco63, as described above in Example 23 and subsequently fusing the two fragments by PCR to assemble the full length gene. The following PCR primer combinations were used:

fragment 1:

Primer 1: EcoRIHBcAg(s) (see Example 23)

Primer 2: Lys-HBcAg(as) (see Example23)

fragment 2:

Primer 3: Lys-HBcAg(s) (see Example23)

Primer 4: HBcAgwtHindIIII

CGCGTCCCAAGCTTCTAACATTGAGATTCCCGAGATTG (SEQ ID NO:386)

Assembly:

Primer 1: EcoRIHBcAg(s) (see example 23)

Primer 2: HBcAgwtHindIIII

The assembled full length gene was then digested with the EcoRI (GAATTC) and HindIII (AAGCTT) enzymes and cloned into the pKK vector (Pharmacia) cut at the same restriction sites.

EXAMPLE 52

Coupling of muTNFa Peptide to HBcAg1-183Lys and Immunization of Mice with Vaccines Comprising HBcAg1-183Lys - muTNFa Peptide Arrays

A. Coupling of muTNFa Peptide to HBcAg1-183Lys

HBcAg1-183Lys at a concentration of 0.6 mg/ml (29 μM) was treated with iodacetamide as described in Example 32. HBcAg1-183Lys was then reacted with a fifty-fold excess of the cross-linker Sulfo-MBS, as described in Example 32, and dialyzed overnight against 20mM Hepes, pH 7.2, at 4°C. Activated (derivatized) HBcAg1-183Lys was reacted with a five-fold molar excess of the peptide muTNFa (sequence: CGGVEEQLEWLSQR (SEQ ID NO:387)), diluted directly into the HBcAg1-183Lys solution from a 100 mM stock solution in DMSO) at RT for 4 hours. The coupling reaction (about 1 ml solution) was dialyzed against 2x 2 liters of 20mM HEPES pH 7.2, at 4°C, for 4 hours. The dialyzed coupling reaction was frozen in aliquots in liquid nitrogen and stored at –80°C until immunization of the mice.

Please amend the last paragraph on page 196 as follows:

2cysLys-mut HBcAg1-149 was reacted at a concentration of 2 mg/ml for 30 min. at RT with a fifty-fold excess of cross-linker in 20 mM Hepes, 150 mM NaCl, pH 7.2. Excess cross-linker was removed by dialysis overnight, and activated (derivatized) 2cysLys-mut HBcAg1-149 capsid protein was reacted with a ten-fold excess of 3'TNF II peptide (SEQ:

SSQNSSDKPVAHVVANHGVGGC (SEQ ID NO:359), diluted from a 100 mM stock solution in DMSO) for 4 hours at RT. The reaction mixture was then dialyzed overnight in a dialysis tubing with a molecular weight cutoff of 50000 Da, frozen in liquid nitrogen and stored at –80°C until immunization of the mice.

Please amend the last paragraph on page 197 and continuing onto page 198 as follows:

The following Aβ peptides were chemically synthesized: DAEFRHDSGYEVHHQGGC (abbreviated as "Aβ 1-15"; SEQ ID NO:367), a peptide which comprises the amino acid sequence from residue 1-15 of human Aβ, fused at its C-terminus to the sequence GGC for coupling to Qβ capsid protein and CGHGNKSGLMVGGVVIA (abbreviated as "Aβ 33-42"; SEQ ID NO:369) a peptide which comprises the amino acid sequence from residue 33-42 of Aβ fused at its N-terminus to the sequence CGHGNKS (SEQ ID NO:405) for coupling to Qβ capsid protein. Both peptides were used for chemical coupling to Qβ as described in the following.

Please amend the second full paragraph on page 199 as follows:

The following Aβ peptide ("Aβ 1-27"; SEQ ID NO:368) was chemically synthesized DAEFRHDSGYEVHHQKLVFFAEDVGSNGGC . This peptide comprises the amino acid sequence from residue 1-27 of human Aβ, fused at its C-terminus to the sequence GGC for coupling to Qβ capsid protein.

Please amend the specification starting from line 21 on page 204 to line 11 on page 205 as follows:

Plasmids were based on the expression plasmid VAE051-pASK116. This plasmid contains the coding regions for the heavy chain and for the light chain of the mimobody. The following primers were used to introduce cys-containing linkers at the C-terminus of the heavy chain:

Primer CA2F:

CGGCTCGAGCATCACCATCACCATCACGGTGAAGTTAAACTGCAGCTGGAGTCG (SEQ ID NO:388)

Primer CA1R:

CATGCCATGGTTAACCACAGGTGTGGGTTTTATCACAAGATTTGGGCTCAAC (SEQ ID NO:389)

Primer CB1R:

CATGCCATGGTTAACCACACGGCGGAGAGGTGTGGGTTTTATCACAAGATTTGGG CTCAAC (SEQ ID NO:390)

Primer CC1R:

CCAGAAGAACCCGGCGGGGTAGACGGTTTCGGGCTAGCACAAGATTTGGGCTCA ACTC (SEQ ID NO:391)

Primer CC1F:

CGCCGGGTTCTTCTGGTGGTGCTCCGGGTGGTTGCGGTTAACCATGGAGAAAATA AAGTG (SEQ ID NO:392)

Primer CCR2:

CTCCCGGGTAGAAGTCAC (SEQ ID NO:393)

Please amend the specification starting at the last paragraph on page 207 to the fourth full paragraph on page 208 as follows:

The sequence of the light chains of pCA2, pCB2 and pCC2 is the same and as follows:

DIELVVTQPASVSGSPGQSITISCTGTRSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPS
GVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLGVFGGGTKLTVLGQPKAAP
SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY
AASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:394)

The sequence of the heavy chain of pCA2 is:

EVKLQLEHHHHHHGEVKLQLESGPGLVKPSETLSLTCTVSGGSISSGGYYWTWIRQR
PGKGLEWIGYIYYSGSTSYNPSLKSRVTMSVDTSKNQFSLRLTSVTAADTAVYYCAR
ERGETGLYYPYYYIDVWGTGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKRVEPKSCDKTHTCG (SEQ ID NO:395)

The sequence of the heavy chain of pCB2 is:

EVKLQLEHHHHHHGEVKLQLESGPGLVKPSETLSLTCTVSGGSISSGGYYWTWIRQR
PGKGLEWIGYIYYSGSTSYNPSLKSRVTMSVDTSKNQFSLRLTSVTAADTAVYYCAR
ERGETGLYYPYYYIDVWGTGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKRVEPKSCDKTHTSPPCG (SEQ ID NO:396)

The sequence of the heavy chain of pCC2 is:

EVKLQLEHHHHHHGEVKLQLESGPGLVKPSETLSLTCTVSGGSISSGGYYWTWIRQR
PGKGLEWIGYIYYSGSTSYNPSLKSRVTMSVDTSKNQFSLRLTSVTAADTAVYYCAR

ERGETGLYYPYYYIDVWGTGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCASPKPSTPPGSSGGAPGGC (SEQ ID NO:397)

Please amend the specification from the second paragraph on page 211 to the third full paragraph on page 212 as follows:

The Flag peptide, to which a CGG sequence was added N-terminally for coupling, was chemically synthesized and had the following sequence: CGGDYKDDDDK (SEQ ID NO:147). This peptide was used for chemical coupling to wt Qβ capsid protein and the Qβ mutant capsid protein as described in the following.

[[E]]A. Coupling of Flag peptide to Qβ capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-GMBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

B. Coupling of Flag peptide to Qβ–240 capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ–240 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-GMBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2

at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C.

C. Coupling of Flag peptides to Qβ–250 capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ–250 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-GMBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 00 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C.

D. Coupling of Flag peptides to Qβ–259 capsid protein

[[_____]]A solution of 100 [[u]]µl of 2 mg/ml Qβ–259 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-GMBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

Please amend the specification from the second full paragraph on page 213 to the first full paragraph on page 215 as follows:

The Flag peptide, to which a CGG sequence was added N-terminally for coupling, was chemically synthesized and had the following sequence: CGGDYKDDDDK (SEQ ID NO:147). This peptide was used for chemical coupling to wt Qβ capsid protein and the Qβ mutant capsid protein as described in the following.

[[F]]A. Coupling of Flag peptides to Qβ capsid protein

[[____]]A solution of 100 [[u]]µl of 2 mg/ml Qβ capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-MBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C.

B. Coupling of Flag peptide to Qβ–240 capsid protein

[[____]]A solution of 100[[u]]µl of 2 mg/ml Qβ–240 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-MBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The

reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

C. Coupling of Flag peptide to Qβ–250 capsid protein

[[___]]A solution of 100[[u]]µl of 2 mg/ml Qβ–250 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-MBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C.

D. Coupling of Flag peptides to Qβ–259 capsid protein

[[__]]A solution of 100[[u]]µl of 2 mg/ml Qβ–259 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.2 was reacted for 60 minutes with 7 µl of a solution of 65 mM Sulfo-MBS (Pierce) in H_2O at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C. 100 µl of the dialyzed reaction mixture was then reacted with 0.58 µl of 100 mM Flag peptide stock solution (in H_2O) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.2 at 4[[>]]°C.

Please amend the specification from the third full paragraph on page 215 to the third full paragraph on page 216 as follows:

The Flag peptide, to which a CGG sequence was added N-terminally for coupling, was chemically synthesized and had the following sequence: CGGDYKDDDDK (SEQ ID NO:147). This peptide was used for chemical coupling to the Qβ mutants as described in the following.

A Coupling of Flag peptides to Qβ-240 capsid protein

[[__]]A solution of 100 [[u]]µl of 2 mg/ml Qβ-240 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM SMPH (Pierce) in DMSO at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 90 [[□]]µl of the dialyzed reaction mixture was then reacted with 1.3 [[□]]µl of 50 mM Flag peptide stock solution (in DMSO) for two hours at 25°C on a rocking shaker. The reaction

mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

B. Coupling of Flag peptides to Qβ-250 capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ-250 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM SMPH (Pierce) in DMSO at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 90 µl of the dialyzed reaction mixture was then reacted with 1.3 µl of 50 mM Flag peptide stock solution (in DMSO) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

C. Coupling of Flag peptide to Qβ-259 capsid protein

[[_____]]A solution of 100 [[u]]µl of 2 mg/ml Qβ–259 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM SMPH (Pierce) in DMSO at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 90 µl of the dialyzed reaction mixture was then reacted with 1.3 µl of 50 mM Flag peptide stock solution (in DMSO) for two hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

Please amend the specification from the second full paragraph on page 217 to the second full paragraph on page 218 as follows:

A. Coupling of PLA_2-Cys protein to Qβ-240 capsid protein

[[_____]]A solution of 100 [[u]]µl of 2 mg/ml Qβ-240 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM SMPH (Pierce) in DMSO at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 90 µl of the dialyzed reaction mixture was mixed with 146 [[ul]]µl 20 mM Hepes, 150 mM NaCl, pH 7.4 and reacted with 85.7 [[ul]]µl of 2.1 mg/ml PLA_2-Cys stock solution for four hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

B. Coupling of PLA_2-Cys protein to Qβ-250 capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ-250 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM SMPH (Pierce) in DMSO at 25[[>]]°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C. 90 µl of the dialyzed reaction mixture was mixed with 146 [[ul]]µl 20 mM Hepes, 150 mM NaCl, pH 7.4 and reacted with 85.7 [[ul]]µl of 2.1 mg/ml PLA_2-Cys stock solution for four hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]°C.

C. Coupling of PLA_2-Cys protein to Qβ-259 capsid protein

[[___]]A solution of 100 [[u]]µl of 2 mg/ml Qβ-259 capsid protein in 20 mM Hepes. 150 mM NaCl pH 7.4 was reacted for 30 minutes with 2.94 µl of a solution of 100 mM

SMPH (Pierce) in DMSO at 25[[>]]≅°C on a rocking shaker. The reaction solution was subsequently dialyzed twice for 2 hours against 2 L of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]≅°C. 90 µl of the dialyzed reaction mixture was mixed with 146 µl 20 mM Hepes, 150 mM NaCl, pH 7.4 and reacted with 85.7 µl of 2.1 mg/ml PLA_2-Cys stock solution for four hours at 25°C on a rocking shaker. The reaction mixture was subsequently dialyzed 2x 2 hours against 2 liters of 20 mM Hepes, 150 mM NaCl, pH 7.4 at 4[[>]]≅°C.

Please delete the Sequence Listing (pages 1-193) as filed in the Preliminary Amendment and Submission of Substitute Sequence Listing on January 2, 2003, and insert therefor the substitute Sequence Listing (pages 1-221) appended hereto.